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To cite this article: Kyohei Kiyota, Masato Yoshimitsu, Taro Satsuki-Murakami, Kazuhiko Akutsu, Keiji Kajimura & Tetsuo Yamano (2017) Detection of the tomato allergen Sola I 1 and evaluation of its reactivity after heat and papain treatment, Food and Agricultural Immunology, 28:6, 1450-1459, DOI: [10.1080/09540105.2017.1347914](https://doi.org/10.1080/09540105.2017.1347914)

To link to this article: <http://dx.doi.org/10.1080/09540105.2017.1347914>



Published online: 05 Jul 2017.



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Detection of the tomato allergen Sola l 1 and evaluation of its reactivity after heat and papain treatment

Kyohei Kiyota^{ab}, Masato Yoshimitsu^a, Taro Satsuki-Murakami^b, Kazuhiko Akutsu^a, Keiji Kajimura^a and Tetsuo Yamano^a

^aDivision of Hygienic Chemistry, Osaka Institute of Public Health, Nakamichi, Higashinari-ku, Osaka-shi, Osaka, Japan; ^bDivision of Hygienic Chemistry, Osaka Institute of Public Health, Tojo-cho, Tennoji-ku, Osaka-shi, Osaka, Japan

ABSTRACT

To detect the tomato allergen profilin (Sola l 1) from fresh tomato and tomato-containing processed foods, we generated a rabbit polyclonal anti-Sola l 1 peptide antibody. An indirect enzyme-linked immunosorbent assay (iELISA) based on the antibody was established for semiquantification of Sola l 1, and standard curves were generated using Sola l 1 homologue recombinant birch profilin (rBet v 2) as a calibrator. The mean concentration of tomato extracts was 25.6 ± 9.1 $\mu\text{g/g}$, and the concentrations of tomato-containing processed foods ranged from 13.3 to 24.8 $\mu\text{g/g}$. The reactivity of Sola l 1 after heat and papain treatment of tomato extracts was evaluated using competitive iELISA, and the results showed that Sola l 1 reactivity was stable with or without treatment. Our findings suggested that tomato-containing processed foods and fresh tomatoes both contained Sola l 1 and could have the potential to induce allergic symptoms in patients with tomato allergies.

ARTICLE HISTORY

Received 29 May 2017
Accepted 25 June 2017

KEYWORDS

Tomato allergen; profilin; Sola l 1; polyclonal antibody; enzyme-linked immunosorbent assay

Introduction

Tomatoes (*Solanum lycopersicum*) are widely consumed as both fresh and processed foods worldwide and are rich in nutrients, such as lycopene (Abete et al., 2013), which may provide health benefits. Since Japanese individuals consume large amounts of tomatoes, about 9 kg/year (Takada, 2012), the Japanese authorities have established tomatoes as important vegetables for dietary habit (Kanayama, 2017).

Despite their health benefits, tomatoes contain some proteins that can cause allergies. Indeed, tomatoes have been shown to contain seven representative allergens (Sola l 1–7); these allergens are included in the official allergen database of the Allergen Nomenclature Subcommittee of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) (WHO/IUIS, Allergen Nomenclature Sub-Committee, 2017). Among these allergens, Sola l 1, also known as profilin (McKenna et al., 2016), has a highly conserved amino acid sequence among plants (Radauer & Breiteneder, 2007). Patients with allergies to profilin can exhibit cross-reactivity with other profilin

CONTACT Kyohei Kiyota ✉ kiyota@iph.osaka.jp Division of Hygienic Chemistry, Osaka Institute of Public Health, 1-3-69 Nakamichi, Higashinari-ku, Osaka-shi, Osaka 537-0025, Japan

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proteins (Song, Zhang, Liu, & Ran, 2007) and often develop adverse reactions, such as oral allergy syndrome, soon after eating causative foods (Santos & Van Ree, 2011).

Since consuming even small amounts of allergens can induce adverse reactions (Zhu, Pouillot, Kwegyir-Afful, Luccioli, & Gendel, 2015), avoidance of the causative allergen is important in patients with allergies. For this reason, in South Korea, food products containing tomatoes must be appropriately labelled (Han, Kim, & Ahn, 2012). Although patients with tomato allergies have also been encountered in Japan (Kondo, Urisu, & Tokuda, 2002), little is currently known about the content of tomato allergens in fresh tomatoes and food products or how to control them. Therefore, appropriate analytical methods need to be developed to facilitate the detection and control of tomato allergens in food products.

In this study, we produced a rabbit polyclonal antibody against Sola l 1 peptide and assessed the content of Sola l 1 using a newly developed indirect enzyme-linked immunosorbent assay (iELISA) with this anti-Sola l 1 antibody and recombinant birch pollen profilin (rBet v 2) as a calibrator. The reactivity of the peptide after heat and papain treatments was evaluated.

Materials and methods

Production of a polyclonal anti-Sola l 1 peptide antibody

A polyclonal antibody against Sola l 1 was prepared by immunising NZW rabbits intradermally with 0.4 mg of the synthetic peptide, $_3\text{WQTYVDDHLMCD}_{14}$ (GenBank accession no. AAL29690.1, UniProtKB accession no. Q8VWR0; Figure 1), conjugated with keyhole limpet hemocyanin in Freund's complete adjuvant. After the first injection, 0.2 mg of the same immunogen in Freund's incomplete adjuvant was administered to rabbits every 2 weeks. On day 63, following collection of sera, antibodies were purified from antiserum using an affinity column coupled with the same peptide as immunogen, and the aliquots of purified antibodies were stored at -20°C until further use. All experiments were performed by Asahi Glass Co., Ltd. (Tokyo, Japan).

Sample preparation

Fresh tomatoes grown in Japan and other samples were purchased from local supermarkets in Osaka, Japan, and used to prepare extracts. After thorough washing and removal of

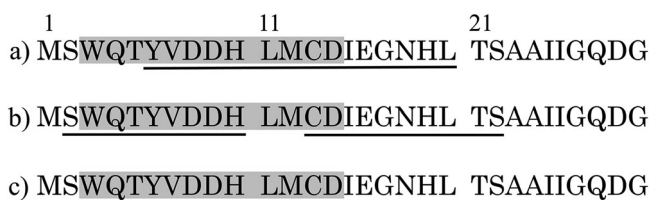


Figure 1. Amino acid residues 1–30 of Sola l 1, highlighting the position of the peptide used as the immunogen. Underlines show the residues previously described as potential IgE epitopes of Sola l 1 homologues, including (a) melon profilin (López-Torrejón et al., 2007), (b) bell pepper and celery profilins (Radauer et al., 2006), and (c) birch pollen profilin (Fedorov et al., 1997).

the hulls, 8 g fresh tomato homogenates was extracted with 8 mL phosphate-buffered saline (PBS, pH 7.4) containing 137 mM NaCl, 2.7 mM KCl, 8.1 mM $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, and 1.47 mM KH_2PO_4 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 0.5 h. Extracts from tomato-containing processed foods were also prepared. Following centrifugation at $15,000 \times g$ and 4°C for 10 min, the supernatants were stored at -20°C as extracts until further use.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting

Tomato extracts (1.5 μg) were denatured at 95°C for 5 min and then loaded on NuPAGE Novex 12% Bis-Tris protein gels (Thermo Fisher Scientific, Waltham, MA, USA) and separated by SDS-PAGE for 35 min at 200 V in 2-(N-morpholino)ethanesulphonic acid (MES) buffer (Thermo Fisher Scientific). After separation, a part of gel was stained with Coomassie brilliant blue (Bio-Rad, Hercules, CA, USA), and the proteins were electroblotted onto polyvinylidene fluoride membranes (iBlot western blotting system; Thermo Fisher Scientific) for 7 min. The membranes were blocked for 1 h with 0.1% bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) in Tris-buffered saline containing 50 mM Tris, 150 mM NaCl, and 0.1% Tween 20 (TBST). Subsequently, the membranes were incubated with 3.1 $\mu\text{g}/\text{mL}$ rabbit anti-Sola l 1 peptide antibody (diluted with blocking buffer) at 20°C for 1 h. After washing three times with TBST for 5 min each, the antibodies were reacted for 1 h with peroxidase-conjugated goat antibodies against rabbit IgG (A6154; Sigma-Aldrich) diluted at 1:8000 with blocking buffer. After washing three times with TBST for 5 min, the reaction with antigen and antibody was detected using a 3,3',5,5'-tetramethylbenzidine peroxidase substrate solution (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA) as a substrate. Tomato extracts were concentrated and dialysed against PBS using Amicon Ultra centrifugal filter devices (NMWL: 3000 Da; Merck, Darmstadt, Germany) for both SDS-PAGE and immunoblotting. The protein concentration was determined using a Bradford assay kit (Bio-Rad).

Semiquantification of sola l 1 by iELISA

iELISA was carried out in flat-bottomed 96-well microplates (Sumitomo Bakelite Co. Ltd., Tokyo, Japan) coated overnight at 4°C with 50 $\mu\text{L}/\text{well}$ of rBet v 2 (Biomay AG, Vienna, Austria) at 31.3–500 ng/mL and the diluted samples in coating buffer (15 mM Na_2CO_3 and 35 mM NaHCO_3 , pH 9.6; Wako Pure Chemical Industries, Ltd.). Wells were blocked for 1 h with PBS containing 20% horse serum (Life Technologies) and were then washed three times with PBS containing 0.05% Tween 20 (PBST). After addition of 50 $\mu\text{L}/\text{well}$ of the anti-Sola l 1 peptide antibody diluted to 3.1 $\mu\text{g}/\text{mL}$ with PBST containing 20% horse serum, the plates were incubated for 1 h. After washing five times with PBST, 50 μL of a biotin-labelled goat anti-rabbit IgG antibody (1:2000; Vector Laboratories, Burlingame, CA, USA) was added. The plates were incubated for 1 h and washed five times with PBST, and streptavidin-conjugated horseradish peroxidase (Vector Laboratories) was then added to each well. The plates were incubated for 1 h and then washed eight times with PBST; next, 3,3',5,5'-tetramethylbenzidine (Thermo Fisher Scientific) was added to each well, and plates were incubated for 10 min in dark. The enzymatic

reaction was stopped with 50 μL of 1 mol/L H_2SO_4 , and the optical density was measured at 450 nm using a microplate reader (MTP-300; Corona Electric Co. Ltd., Ibaraki, Japan). The data were analysed using SF5 software (Corona Electric Co., Ltd.). Each experiment was performed in triplicate.

Sample treatment

Tomato extracts were heated at 37°C, 85°C, or 95°C for 30–90 min. For papain treatment, 100 μL of tomato extracts was added to 100 μL of papain solution (Abe & Ohba, 1998) containing 0.4% w/v papain (Wako Pure Chemical Industries, Ltd.), 1.0% w/v cysteine (Nacalai Tesque, Inc., Kyoto, Japan), and 4 $\times$ PBS. The mixtures (pH 6) were incubated at 50°C for 0.5–3.0 h and immediately denatured at 95°C for 30 min. Samples were stored at -20°C until further use and diluted with PBST containing 20% horse serum for indirect competitive ELISA.

Indirect competitive ELISA

Fifty microlitres of tomato extracts diluted at 1:100 with coating buffer was coated onto flat-bottomed 96-well microplates (Sumitomo Bakelite Co. Ltd.) overnight at 4°C, and the plates were then blocked for 1 h with PBS containing 20% horse serum (Life Technologies). After washing three times with PBST, 25 μL of heat- or papain-treated tomato extracts plus 25 μL anti-Sola l 1 peptide antibody (6.2 $\mu\text{g}/\text{mL}$) were added, and the plates were incubated for 1 h. The plates were then washed again with PBST, and 50 μL of a biotin-labelled goat anti-rabbit IgG antibody (1:2000; Vector Laboratories) was added; plates were then incubated for 1 h, and the procedures described above were performed to obtain and analyse the data. Each experiment was performed in duplicate.

Results and discussion

Antibody characterisation

In order to develop the iELISA, we produced rabbit polyclonal anti-Sola l 1 peptide antibodies, which could detect Sola l 1 from tomato extracts and the Sola l 1 homologue rBet v 2 as a band at approximately 14 kDa by immunoblotting (Figure 2).

Although polyclonal anti-recombinant or purified protein antibodies have multiple epitopes, making it difficult to identify the specific epitopes, this was not a challenge for the antibody produced in this study because a short peptide was used for immunisation. Since little was known about the human IgE epitopes of Sola l 1, we designed a synthetic peptide, $_3\text{WQTYVDDHLMCD}_{14}$, as an immunoantigen using online resources (<http://rnaidesigner.thermofisher.com/peptide/design.do>); this peptide sequence overlaps with the human IgE epitope of Sola l 1 homologue allergens (Fedorov, Ball, Mahoney, Valenta, & Almo, 1997; López-Torrejón et al., 2007; Radauer et al., 2006) (Figure 1). Therefore, we assumed that the site may be important for the allergenicity of Sola l 1. Because the amount of IgE was too low to develop an ELISA for Sola l 1 quantification and antigenicity evaluation, the produced antibody was a good tool to achieve the aims of this study.

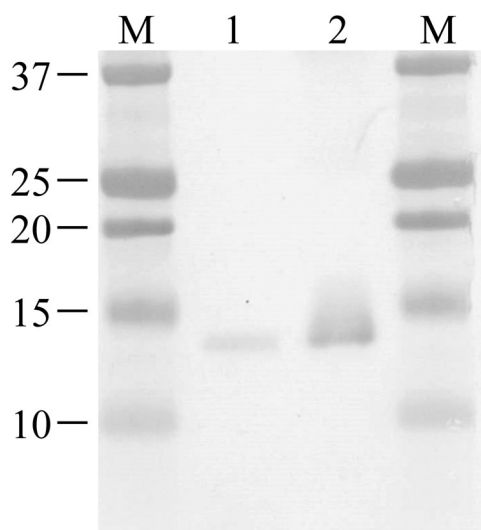


Figure 2. Immunoblotting results with the polyclonal anti-Sola I 1 peptide antibody. Lane 1, rBet v 2; lane 2, tomato extract; M, molecular weight marker (kDa).

Parameters of indirect ELISA based on the anti-sola I 1 peptide antibody

A four-parameter logistic standard curve was generated using rBet v 2 with two-fold serial dilutions (range: 31.3–500 ng/mL; [Figure 3](#)). The limit of detection was 31.3 ng/mL, with a coefficient of variation (CV) of less than 30%, and the quantification range was determined to be from 62.5 to 500 ng/mL, with a CV of less than 10% in 11 experiments, as previously reported (Kiyota et al., 2017).

Measurement of Sola I 1 concentrations

Sola I 1 concentrations in fresh tomato extracts and tomato-containing processed foods were determined by iELISA, and the results are summarised in [Table 1](#). The amount of

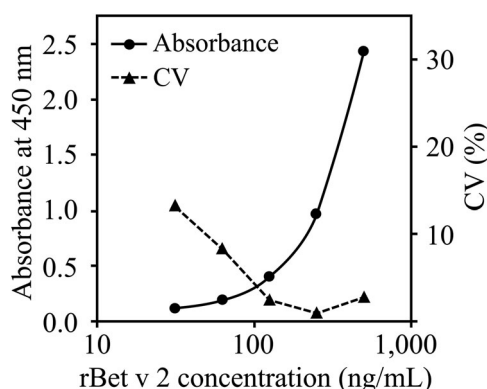


Figure 3. Standard curve and the CV of iELISA. Solid line: a typical standard rBet v 2 quantification curve. Dotted line: CV of values calculated from the standard curves at each concentration. Limit of detection: 31.3 ng/mL (CV <30%); limit of quantitation; 62.5 ng/mL (CV <10%), respectively.

Table 1. Sola l 1 levels in fresh tomatoes and tomato-containing processed foods.

Foods	Manufacturer No. ^a	Sola l 1 concentration (µg/g) ^b
Fresh tomato (1:100 dilution, <i>n</i> = 5)	–	25.6 ± 9.1
Tomato juice (1:100 dilution, <i>n</i> = 1)	1	13.3
	2	20.6
	3	16.8
	4	16.5
	5	15.9
	6	14.4
	7	14.6
Puree (1:100 dilution, <i>n</i> = 1)	1	21.8
	8	24.8

Limit of quantification: 6.25 µg/g in the rBet v 2 dose-response curve. –: Not available.

^aNumbers show the different manufacturers.

^bTomato profilin (Sola l 1) concentration, mean ± SD.

Sola l 1 in fresh tomatoes ranged from 18.0 to 40.4 µg/g, with a mean concentration of 25.6 ± 9.1 µg/g; this value was estimated to be more than 10 times higher than that in fresh oranges (Kiyota et al., 2016). In tomato-containing processed foods, such as juices and purees, Sola l 1 levels ranged from 13.3 to 24.8 µg/g.

The amino acid sequence homology of rBet v 2 with Sola l 1 is about 75%, and profilin is useful to diagnose tomato allergies (Asero, 2013), which implies that they have common epitopes. Thus, the results of this study were obtained using rBet v 2 as a calibrator, as has been used in previous reports (Abedini et al., 2011; Ahrazem et al., 2007; Alvarado et al., 2014; Kiyota et al., 2016). In order to accurately measure the Sola l 1 concentration in the samples, corresponding recombinant or purified Sola l 1 should be prepared as standards of the calibration curve.

Since tomatoes require profilin expression for growth (Le et al., 2010), Sola l 1-deficient tomatoes are practically unavailable, although Le et al. (2010) have described the development of hypoallergenic and transgenic tomatoes expressing non-allergenic yeast profilin. Therefore, to avoid consumption of foods inducing allergic symptoms, it is necessary to control tomato allergen ingestion. Moreover, although no studies have reported Sola l 1 levels in tomatoes, Westphal et al. (2004) reported that Sola l 1 is a tomato allergen, and this assertion has been confirmed by WHO/IUIS. In this study, we showed that Sola l 1 was detected in both fresh tomatoes and tomato-containing processed foods, and the newly developed iELISA will help control Sola l 1 levels during food manufacturing. Although tomato juices and purees are treated by food processing, such as sterilising and filtration, they contain slightly lower amounts of Sola l 1 than fresh tomatoes. Therefore, we tested the reactivity after food processing in subsequent studies.

Reactivity of Sola l 1 after heat and papain treatment

Heat treatment can reduce the allergenicity of some plant food allergens by denaturing and aggregating IgE epitopes (Masthoff et al., 2013; Mejrhith et al., in press; Uberti et al., 2015), whereas other allergens are heat stable (Amnuaycheewa & de Mejia, 2010; Ballmer-Weber et al., 2002). In addition, papain treatment can also reduce allergenicity by digesting IgE epitopes (Liu, Luo, & Li, 2012).

Because sterilisation treatment of tomato juice in Japan requires incubation at 85°C for 30 min or more (Japan External Trade Organization, 2017), we treated tomato extracts at

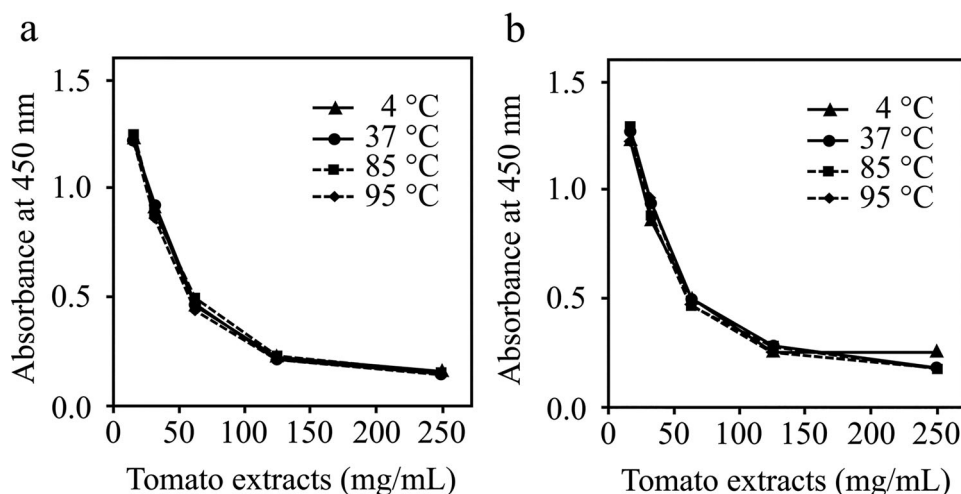


Figure 4. Reactivity of tomato extracts after heat treatment at 4–95°C for (a) 30 min and (b) 90 min was evaluated using indirect competitive ELISA. Average values of absorbance were plotted. The tests were performed in duplicate ($n = 2$).

temperatures of 4–95°C for 30–90 min and then assessed the reactivity of Sola l 1 using indirect competitive ELISA. The results showed that the reactivity of Sola l 1 in heated tomato extracts was stable, regardless of temperature or time (Figure 4). Although we did not test samples treated under high pressures, Sola l 1 may be detectable in tomato-containing processed foods obtained from other stores. Our results suggested that individuals suffering from tomato allergies because of Sola l 1 should be cautious when eating both fresh tomatoes and tomato-containing processed foods.

Next, we tested reactivity after papain treatment (Figure 5). Our findings revealed that there were no dramatic changes in the reactivity in the samples after this treatment,

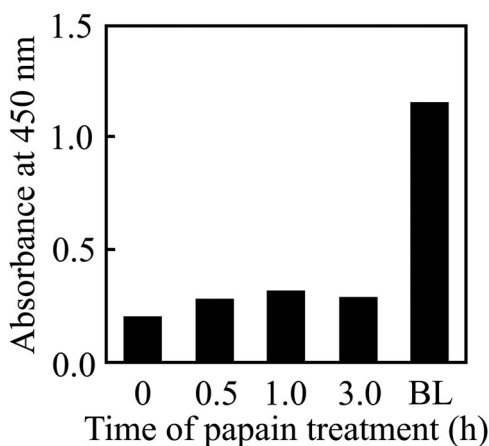


Figure 5. Reactivity of tomato extracts after papain treatment at 50°C for 0–3.0 h was evaluated using indirect competitive ELISA. BL (blank test): PBS was tested instead of tomato extracts. The bar graph shows average values of absorbance. The tests were performed in duplicate ($n = 3$).

suggesting that the epitope of the anti-Sola 1 1 antibody was stable in the presence of papain. The effects of other food-grade proteases (Li, Yu, Goktepe, & Ahmedna, 2016; Susanna & Prabhasankar, 2015) on the digestion of IgE epitopes should also be analysed in order to expand food choice and improve quality of life in patients with tomato allergies. However, since IgE binding tests do not always correlate the diagnosis of food allergies (Muraro et al., 2014; Urisu et al., 2014), oral food challenge tests (Urisu et al., 2014) are required to reliably confirm the reduction of allergenicity in further studies.

Acknowledgements

The authors would like to thank Mikiya Kitagawa for providing valuable advice and technical support.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported in part by a grant from Daido Life Welfare Foundation, Japan.

Notes on contributor

Kyohei Kiyota is a researcher at Division of Hygienic Chemistry, Osaka Institute of Public Health, Japan, who is interesting in the research area; analysis of food allergens with ELISA and PCR systems.

Masato Yoshimitsu is a researcher at Division of Hygienic Chemistry, Osaka Institute of Public Health, Japan. His interest includes food chemistry and analytics of food allergens and pesticides.

Taro Satsuki-Murakami is a Ph.D. in human life science and a researcher at Division of Hygienic Chemistry, Osaka Institute of Public Health, Japan. His interest includes food chemistry and analytics of food allergens.

Kazuhiko Akutsu is a Ph.D. in pharmaceutical science and a researcher at Division of Hygienic Chemistry, Osaka Institute of Public Health, Japan. His interest includes food chemistry and analytics of environmental pollutants and pesticides.

Keiji Kajimura is a Ph.D. in pharmaceutical science and is working at Division of Hygienic Chemistry, Osaka Institute of Public Health, Japan. His interest includes food chemistry.

Tetsuo Yamano is a Ph.D. in veterinary medicine and is working at Division of Hygienic Chemistry, Osaka Institute of Public Health, Japan. His interest includes physical and chemical science.

ORCID

Kyohei Kiyota  <http://orcid.org/0000-0002-6860-7074>

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